Analysis of fats and oils by SFE and SFC

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Supercritical fluids (SCF) have attracted much attention over the past 20 years with regard to their potential application in chemical engineering, industrial processing and environmental remediation. These fluids, particularly supercritical carbon dioxide (SC-CO_2), permit extraction and processing operations to be conducted at relatively low temperatures, using nontoxic and inert gases. The resultant products (both extract and substrate) from these SCF-based processes are solvent-free and minimally altered or degraded during the extraction process. Such factors have served as the basis for the special applications of these fluids in the food industry (1).

Since the early 1980s, there has been a renewed interest in the analytical applications of SCF (2). This has largely been due to the development of suitable analytical equipment for conducting supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) on a routine basis (3). Analytical SFE and SFC continue to be developed to meet the widespread demands of many analysts in the food, environmental and energy-related industries (4). These developments are accelerated by new government regulations regarding the generation, use and disposal of hazardous solvents in the laboratory environment (5).

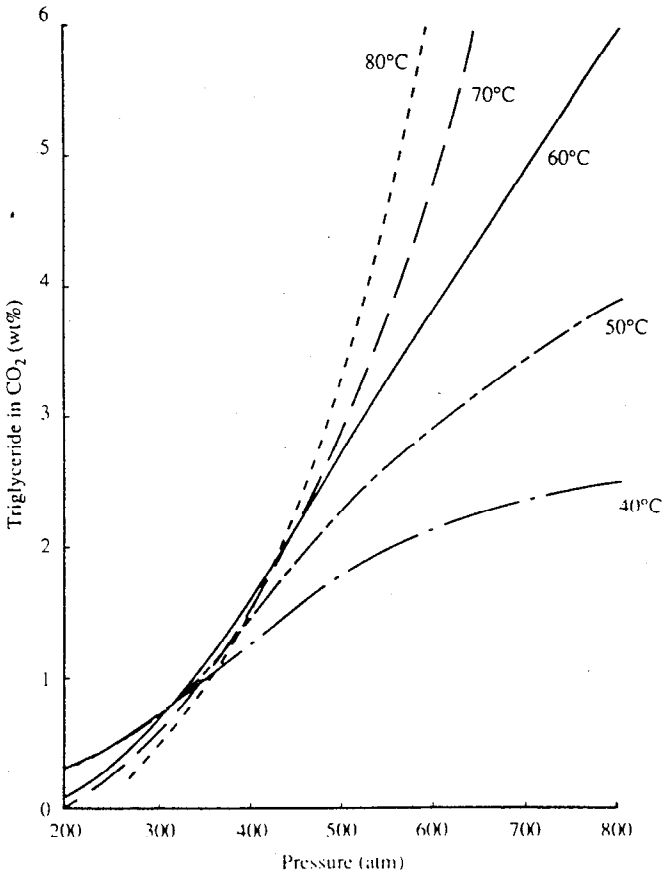

Figure 1. Solubility of soybean oil triglycerides in SC-CO_2 as a function of pressure

Regardless of the scale of the SFE or SFC operation, certain fundamental principles apply. An SCF can be viewed as a unique state of matter, intermediate between a liquid and a gas, whose physical properties are determined by the external pressure and temperature that are applied to the fluid. If the fluid is held at a temperature and pressure above its critical point (T_c and P_c, respectively), then it is said to be in the SCF state, and its density under such conditions can be varied substantially by increasing the applied pressure on the system. Suffice it to say that at high densities such fluids take on the solvent-like properties of many organic solvents and have the capability to dissolve a variety of substances, just as normal liquids do.

Why then should SCFs be of particular interest to the fats and oils analyst? The answer lies partly in the extraordinarily high solubility exhibited by lipid materials in SCF, particularly CO_2, which readily solubilizes nonpolar solutes (6). Such a trend is illustrated by Figures 1 and 2, where the solubility of soybean oil triglycerides as a function of CO_2 pressure (7) is plotted. For the illustrated isotherms, it is possible to obtain lipid solubilities ranging from a few weight percent to over 25 wt%, depending on the pressure and temperature conditions chosen. Such solubilities are more then sufficient for chromatography under SCF conditions, the exhaustive delipidation of fat- and oil-containing samples by SFE, and the

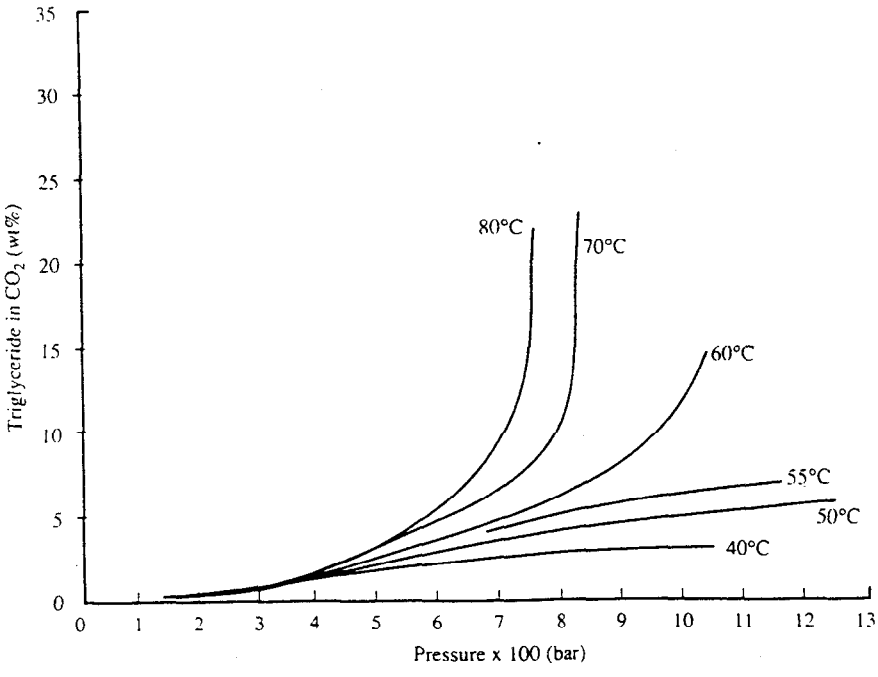

Figure 2. Solubility of soybean oil triglycerides in SC-CO_2 at high pressure

partial fractionation of lipid moieties by SFE or SFC.

Other lipids, such as fatty acids, tocopherols, sterols, etc., exhibit similar solubility trends as those depicted in Figures 1 and 2 (8,9). Unfortunately the high overall solubility of many lipid compounds in SCF compromises the molecular specificity of SFE in the absence of an auxiliary technique, such as chromatography (10). However, the use of lower pressures and/or temperatures permits SCF to be applied to many analytical applications that do not require such high finite lipid solubilities, such as capillary SFC, residue analysis and on-line SFE. We shall now examine some of these applications to illustrate the usefulness of analytical SFE and SFC in applied lipid analysis.

Off-line SFE

Mechanistically, analytical SFE is applied in either an off- or on-line mode. Off-line extraction usually implies that the sample of interest is extracted in a discrete operation in which the extract is first isolated and then independently analyzed by any one of a variety of techniques. Within limits, the extraction and temperature and pressure can be varied to control the composition of the lipid extract; however, it is common when extracting lipid matter from different sample matrices to do an exhaustive extraction. However, even when performing extractions at high pressures and temperatures (700 bar, 80°C), an excellent separation can be achieved between phospholipids and nonpolar lipids (11). The former can easily be solubilized in SCF by the addition of a cosolvent, such as ethanol or methanol to the SC-CO_2.

An example of a selective extraction of interest to the lipid analyst is the isolation of cholesterol from oil or fat matrix, such as cod liver oil matrix (12). Adjustment of the CO_2 density to 0.40 g/mL (120 atm, 60°C) allows the cholesterol to be isolated from the oil as shown in Figure 3. A higher CO_2 density, 0.93 g/mL (350 atm, 40°C), permits extraction of the triglycerides, as indicated by the upper

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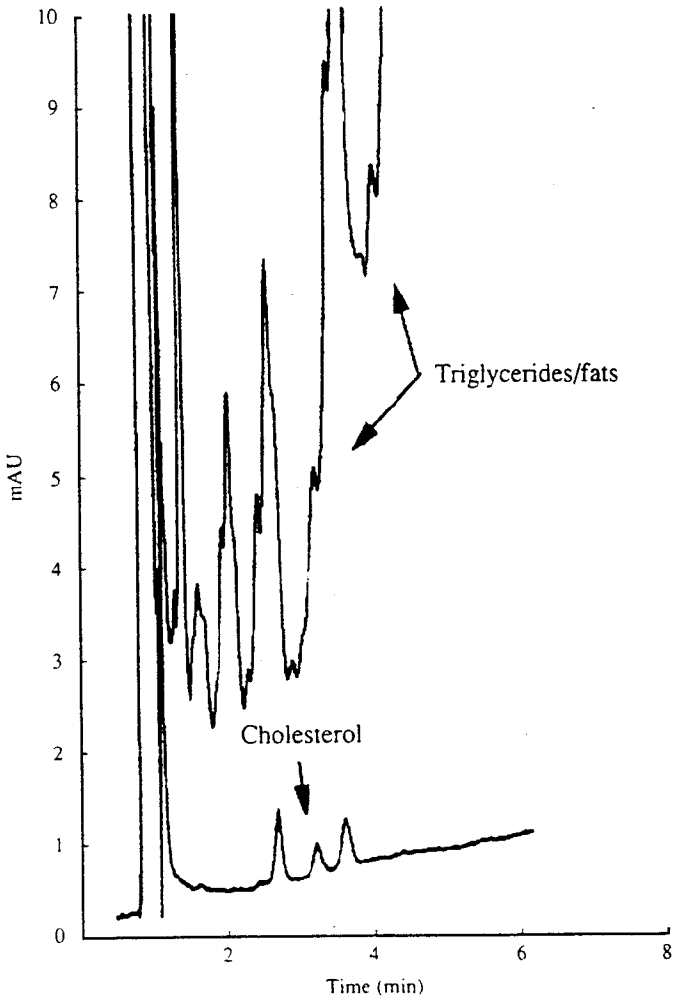

Figure 3. HPLC of SFE fractions of cod liver oil

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ultraviolet detector trace (210 nm) from high-performance liquid chromatographic analysis of the two extracts (Fig. 3). This simple fractionation was accomplished on a Hewlett-Packard Model 7680A SCF extractor, using a CO_2 flow rate of 4 mL/min for 10 min, after an initial static hold of 1 min. The cod liver oil sample (500 µL) was initially mixed with a diatomaceous earth sorbent.

Off-line SFE preparation and analysis of multiple samples is currently available, due in part to the initial pioneering efforts of researchers at NCAUR (13). Rapid SFE (15 min) of lipid phases in pesticide-containing meat products could be affected simultaneously in six samples by using the apparatus shown in Figure 4. Quantitative lipid and pesticide extractions were obtained at 340–680 atm at 60°C using 5–10 L/min CO_2 flow rates (ambient conditions). Today, commercial instrumentation exists that will permit the analysis of up to 8, 24 or 44 samples, either simultaneously or in a serial mode of extraction.

One particular application of off-line SFE deserves special mention: the determination of fats and oils levels in raw materials and/or processed food products. This application is becoming critically important as analysts seek an alternative to the classic ("ancient") and often-varied Soxhlet extraction technique, which utilizes organic and sometimes flammable and carcinogenic solvents. There remains little doubt that analytical SFE can yield equivalent results to extractions on the same sample using nonpolar organic solvents. This recently has been demonstrated by researchers at NCAUR for the quantitative extraction of oil from three different oilseed types (14).

Perhaps of more interest are the recent results for the extraction of fat from different food matrices by Hopper (as cited in 15) as noted in Table 1. In this case, the SFE results were determined by a simultaneous multi-sample SFE, using a instrument designed for large samples that is a prototype of an earlier unit developed by researchers at NCAUR (13). Note that the SFE results from this off-line technique using high pressure CO_2 are comparable to two methods using liquid solvents. Method 960.39 is a Soxhlet extraction using petroleum ether as specified by the AOAC International (16), while the results in the column labeled PAM 1 are a sequential solvent extraction using ethyl ether, as designated in the Pesticide Analytical Manual of the FDA (17). Despite these encouraging results, collaborative studies need to be undertaken to verify the reproductivity of the SFE method, particularly in lieu of its importance in nutrient analysis (18).

On-line SFE

In contrast to off-line SFE, on-line SFE involves conducting an extraction, followed by transfer of the extract to the analysis instrument, all in a sequential fashion. The analysis instrument of choice is frequently a gas or liquid chromatograph, followed by mass or infrared spectrometry for identification of the separated compounds. The technique has certain advantages and disadvantages, which are worth enumerating.

On-line SFE frequently requires the use of switching and sampling valves to transport the extract from the extraction stage to the analysis step. The extract is deposited and concentrated during a defined period of

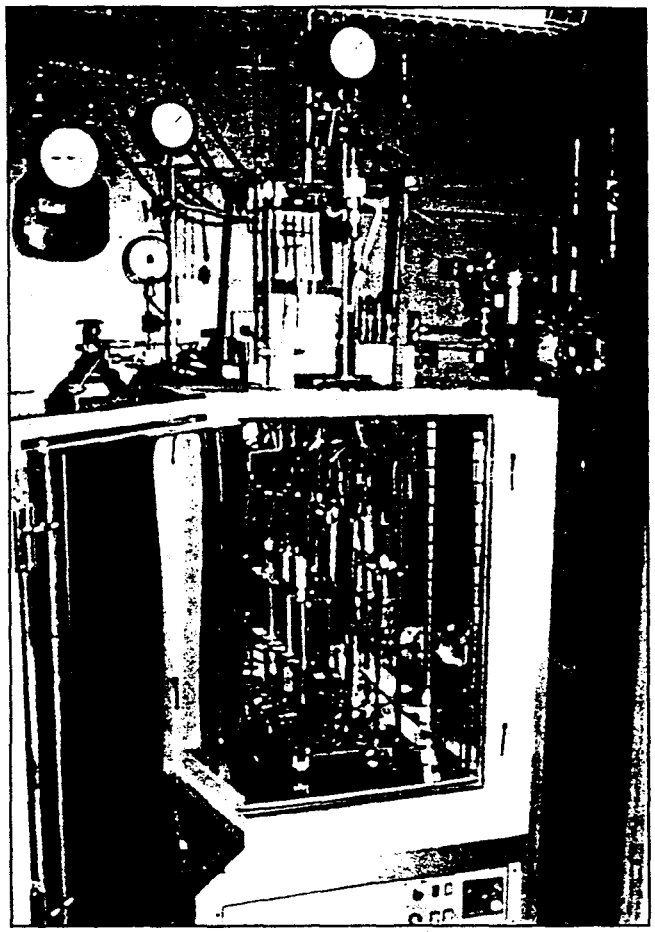

Figure 4. NCAUR multi-sample SCF extractor

extraction, either on some form of a retention gap or at the head of the chromatographic column. This prevents contamination of and loss of the extract prior to the analysis step.

However, the analyst employing on-line SFE also loses the freedom to choose the analytical method once the extraction module is fixed in the system. Considerable "replumbing" may be necessary to mate the SFE step with an alternative analytical technique. The sample sizes that can be analyzed by on-line SFE are often small (mg) in the case of lipid-containing substrates, since the high solubility of lipid compounds in the SCF tends to lead to column overloading in the case of microbore and capillary columns. The careless handling of extraction cells can also lead to analysis artifacts, such as lipids from fingerprints, which show up on the resultant chromatograms.

However, the ability of on-line SPE to extract small samples for subsequent analysis is also an attractive

Table 1
Percent fat extracted (%RSD, n = 6)

Sample	960.39	PAM 1	SFE
Pork sausage	30.55 (4.75)	29.83 (1.55)	29.83 (1.32)
Peanut butter	50.32 (0.39)	49.29 (0.60)	49.51 (0.44)
Cheddar cheese	33.85 (3.13)	33.94 (3.46)	33.26 (1.14)
Corn chips	31.32 (0.62)	31.80 (0.76)	31.51 (0.46)

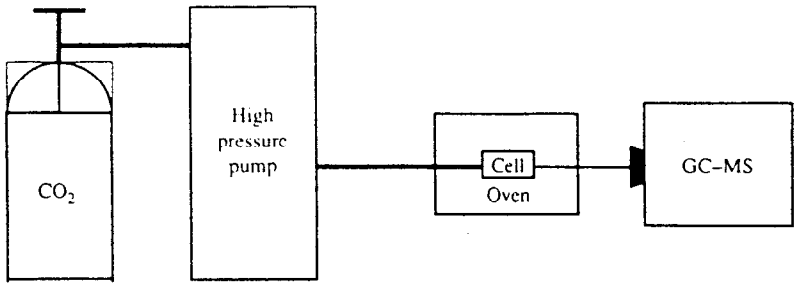

Figure 5. Schematic of SFE/GC/MS system for volatile analysis

feature of the technique. King (19) has shown that the lipid content of single seeds and insects can be characterized by coupling on-line SFE with SFC. Volatiles and semi-volatile components from the degradation of fats and oils also can be identified and quantitated by coupling on-line SFE with SFC or gas chromatography. Recent studies by Snyder (20) have shown that low temperature/pressure extraction of volatiles/semi-volatiles from as little as 10 µL of oxidized oil offers an improved method of characterizing the oil decomposition products. The experimental apparatus is very simple, as depicted in Figure 5, consisting of a high pressure syringe pump in-line with a thermostatted macro-extraction cell connected to the injection part of gas chromatograph. Gentle extraction at 102 atm and 50°C limits the extraction of high molecular weight components and minimizes the decomposition of hydroperoxides to lower molecular weight artifacts, thereby providing a more accurate analysis of volatile components in the oxidized oil.

Recently on-line SFE has been combined with a precolumn chemical reaction followed by gas and/or SFC for the analysis of reaction products. Such a technique allows the study of reactions in SCF media, but perhaps more importantly, the analysis of analytically-useful derivatives. Berg and co-workers (21) have synthesized both methyl and butyl esters from interesterification of triglycerides in edible fats using an immobilized lipase in the extraction cell at 150 atm and 50°C. Similar analytically-useful results have been obtained by King and co-

workers (22), using an aluminum oxide sorbent in the extraction cell for the production of methyl esters. Partial conversion to methyl esters was obtained at 200 atm and 40°C on single plant seeds, thereby allowing seed viability to be maintained after on-line analysis of the methyl esters by gas chromatography.

SFC

SFC offers the lipid analyst some very interesting options that are not easily achieved by using other types of chromatography. Unfortunately SFC is often perceived as a technique that is applicable to only a few niche applications that cannot be solved by gas chromatography (GC) or high-performance liquid chromatography (HPLC). This is not true when one examines the versatility of SFC in applied lipid analysis, as well as advantages of the technique itself.

The uniqueness of SFC-based separations derives in part from the ability of the analyst to vary the mobile phase solvating power as a function of pressure. Hence, many separations in SFC are affected in a similar manner to gradient elution techniques in HPLC, where retention and separation are altered by varying the composition of the mobile phase. SFC utilizes both capillary and packed columns for lipid analysis (23), the latter option being capable of producing very high column efficiencies (24) by using ultrasmall diameter columns. Flame-ionization detection has been the most successful method to date in the analysis of lipids by SFC; however, recent advances in coupling the evaporative light-scattering detector with SFC have been successful (25). Both detectors also offer a universal mode of detection that is not readily available with HPLC.

Several advantages attend the use of SFC that are missing in GC and HPLC. The use of mobile phase pressure/density-programming techniques can eliminate the need for sample preparation prior to analysis (26), since unwanted or interfering components can be injected along with the target analyses and simply eluted out of column by increasing the density of the mobile phase. The relatively benign conditions employed in SFC make it a technique that is compatible for the chromatography of nonvolatile, but thermally labile compounds, or moieties prone to oxidation. The ability of SFC to analyze lipid-type compounds approaching 1000 Daltons in molecular weight also eliminates the need for derivatization, as required in many GC-based methods. SFC also eliminates or reduces the use of solvents relative to HPLC methods.

What are the unique applications of SFC that are of interest to the applied lipid analyst? As noted above, SFC often can be directly applied to an analysis situation without resorting to sample preparation or derivatization. Generic applications include the direct characterization of raw materials or reaction mixtures, the deformulation of commercial products containing a wide range of lipid types and the direct detection of product adulteration or deterioration. Examples of these applications have been provided by King (19).

SFC is also unique in its ability to separate oligomeric mixtures of polymers, surfactants and other homologous series of compounds. The high resolving power of capillary SFC for these applications is due in part to the analyst's ability to specify complex pressure, density or temperature programs which facilitate the separation of oligomeric mixtures. Figure 6 demonstrates this type of separation by SFC for an oleic acid-esterified propoxylated glycerol having 5 moles of propylene oxide/mole of glycerol (low caloric fat substitute), which was obtained by using a asymptotic programmed density ramp from 0.12 to 0.61 g/mL (27).

Other analysts have also employed SFC to great advantage for the characterization of surfactant mixtures (28) and synthetic oligomers (29). Chester (30) recently advocated even higher pressures in SFC to allow the separation of the higher molecular weight species in a oligomeric mixture. Applying this concept along with the choice of the right stationary phase results in an optimal separation of the higher oligomers in a synthetic mixture of ethoxylated steryl alcohol oligomers (Brij 78), as shown in Figure 7.

As in SFE, SFC can provide a general assessment of the lipid components in a natural product matrix, either by coupling SFE with SFC or by simply performing a solvent extraction on the sample matrix, followed by injection into the SFC. An

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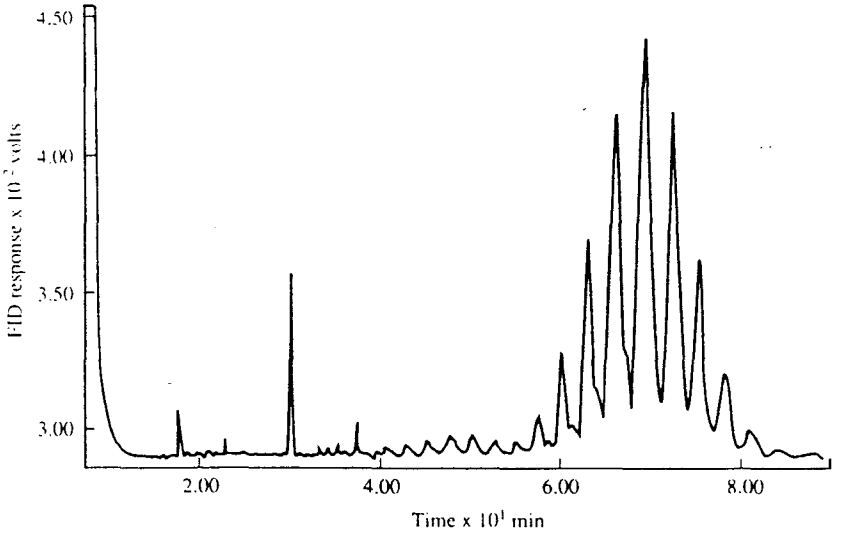

Figure 6. SFC of oleic acid-esterified propoxylated glycerol (EPG) with 5 moles of propylene oxide/mole of glycerol

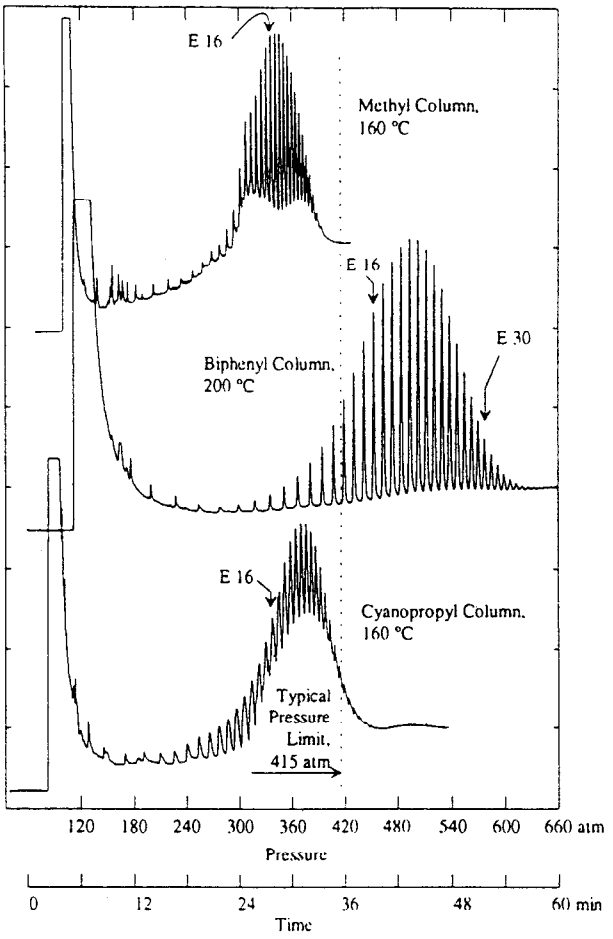

Figure 7. Advantage of high pressure for the SFC analysis of Brij 78. Time axis is for the biphenyl chromatogram.

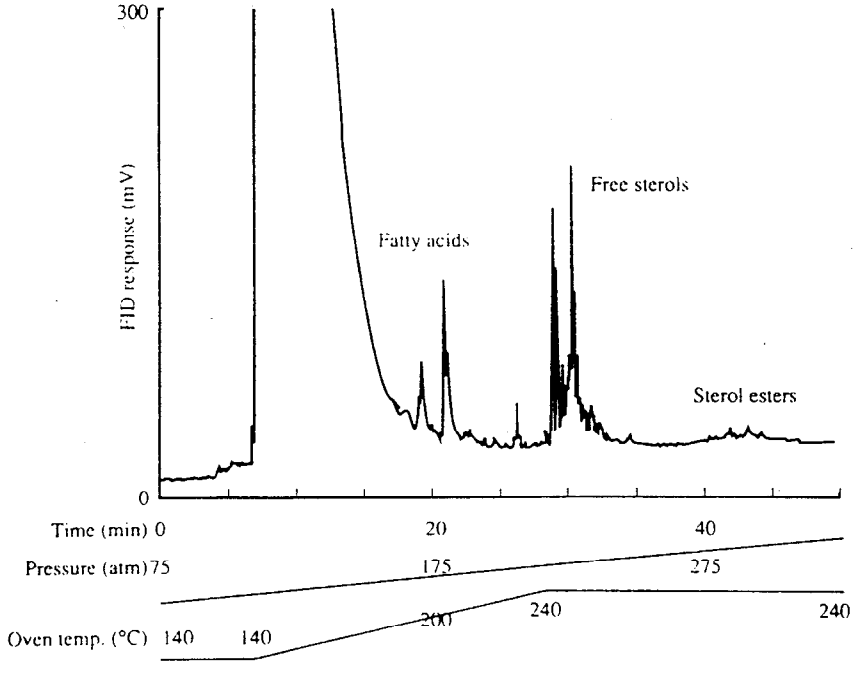

Figure 8. SFC separation of the lipid extract from freeze-dried hamster feces (with permission of *Journal of High Resolution Chromatography*).

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example of this type of analysis as applied to a study on the absorption of dietary fats is illustrated in Figure 8, where the lipid components in hamster feces have been separated by SFC (31). The separation depicted in Figure 8 was accomplished by superimposing both a temperature and pressure gradient during the SFC run to effect a better separation between the sterol esters and triglycerides. Similar SFC profiles could be obtained from either making a liquid injection of a Soxhlet extract or by inserting a fece pellet into an on-line SFE module attached to the SFC.

Maturity of an analytical technique can often be assessed by its application to quantitative or collaborative types of analysis. Routine and standard methods based on SFC have emerged recently and offer not only improvements in analytical methodology, but a reduction in solvent disposal or regeneration costs. Recently the status of the official AOCS method for α-monoglycerides has been noted in *INFORM* (32) and alternative methodology suggested. SFC also can be applied for monoglyceride determination, and recent quantitative studies on commercial emulsifiers indicate that excellent results can be obtained. Table 2 compares the results for total monoglycerides in a commercial emulsifier determined by HPLC using evaporative light-scattering detection, GC of the propionyl ester and SFC with flame-ionization detection on the underivatized sample (33). The agreement between all three methods is excellent.

Future horizons

The future application and potential of SFE and SFC appears promising, since regulatory concerns involving the use and disposal of hazardous solvents opens up a new vista for the above techniques. The above uses also will be accelerated by the federal Nutritional Labeling and Education Act of 1990, where concern over the lipid content of food will assure new uses for SFE and SFC.

On the horizon are some new applications of SCFs, which contain

Table 2
Comparison of monoglyceride results for a commercial emulsifier as determined by the HPLC–ELSD, GC and SFC methods[a]

	Total monoglycerides, g/100 g		
	HPLC–ELSD	**GC/derivatized**	**SFC/underivatized**
Lot 1840			
Mean	92.5	92.3	93.4
%RSD (n)	1.1 (4)	1.3 (4)	3.5 (11)
Lot 6022			
Mean	94.7	94.0	95.9
%RSD (n)	1.5 (4)	1.8 (4)	3.4 (12)

[a]Abbreviations: HPLC–ELSD, high-performance liquid chromatography–evaporative light-scattering detector; GC, gas chromatography; SFC, supercritical fluid chromatography; RSD, relative standard deviation.

elements of both analytical and process technologies. SFE techniques will undoubtedly be coupled with numerous forms of chromatography for further fractionation of the derived extracts and with techniques such as immunoassay (34) for the rapid screening of many samples. The renaissance of SFC indicates the increased use of packed-column methodology, for both capillary and microbore columns, as well as for enhanced selectivity using modest cosolvent addition.

References

1. Rizva, S.S.H., J.A. Daniels, A.L. Benado and J.A. Zollweg *Food Tech. 40 (7):* 57 (1986).
2. Lee, M.L., and K.E. Markides, *Analytical Supercritical Fluid Chromatography and Extraction*, Chromatography Conferences Inc., Provo, Utah, 1990.
3. Wenclawiak, B., *Analysis with Supercritical Fluids: Extraction and Chromatography*, Springer-Verlag, Heidelberg, 1988.
4. Hawthorne, S.B., *Anal. Chem. 62 (11):*633A (1990).
5. Katauskas, T., and H. Goldner, *Res. Dev. 33(4):*40 (1991).
6. Stahl, E., K.W. Quirin and D. Gerard, *Dense Gases for Extraction and Refining*, Springer-Verlag, Heidelberg, 1988, pp. 85–94.
7. Friedrich, J.P., U.S. Patent 4,466, 923 (1984).
8. King, J.W., *J. Am. Oil Chem. Soc. 60:*711 (1983).
9. Chrastil, J., *J. Phys. Chem. 86:*3016 (1982).
10. Favati, F., and J.W. King, *Abstracts of the 4th International Symposium on Supercritical Fluid Chromatography and Extraction*, Cincinnati, Ohio, May 20–22, 1992, pp. 71–72.
11. Friedrich, J.P., G.R. List and A.J. Heakin, *J. Am. Oil Chem. Soc. 59:*288 (1982).
12. Gere, D.R., L.G. Randall, C.R. Knipe, W. Pipkin and L.C. Doherty, *Proceedings of the 9th Annual Waste Testing and Quality Assurance Symposium*, Alexandria, Virginia, July 12–16, 1993.
13. King, J.W., J.H. Johnson, S.L. Taylor, W.L. Orton and M.L. Hopper, *Abstracts of Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy*, Chicago, Illinois, March 4–8, 1991, Abstract No. 180.
14. Taylor, S.L., J.W. King and G.R. List, *J. Am. Oil Chem. Soc. 70:*437 (1993).
15. Sawyer, L.D., *J. Assoc. Off. Anal. Chem. 76(1):*144 (1993).
16. McNeal, J.E. in *Official Methods of Analysis of the AOAC—15th Ed., Vol. 2*, edited by K. Helrich, AOAC International, Arlington, Virginia, 1990, Method 960.39, pp. 931–932.
17. *Pesticide Analytical Manual (PAM)—FDA*, Vol. 1, National Technical Information Service, Springfield, Virginia, 1989, Methods 211.13 (c), (f) and (k).
18. Anonymous, *INFORM 4:*562 (1993).
19. King, J.W., *J. Chromatogr. Sci. 28:*9 (1990).
20. Snyder, J.M., *INFORM 4:*543 (1993).
21. Berg, B.E., E.M. Hansen and T. Greibrokk, *Abstracts of the 2nd European Symposium on Supercritical Fluid Chromatography and Extraction*, Huethig-Verlag, Heidelberg, 1993, pp. 194–202.
22. King, J.W., J.E. France and J.M. Snyder, *Fresenius J. Anal. Chem. 334:* 474 (1992).
23. Markides, K.L., and M.L Lee, *SFC Applications*, Brigham Young University Press, Provo, Utah, 1988, 1989.
24. Chester, T.L., in *Analytical Instrumentation Handbook*, edited by G.W. Ewing, Marcel Dekker Inc., New York, 1990, pp. 843–881.
25. Demirbuker, M., *Analysis of Lipids by Supercritical Fluid Chromatography*, Ph.D. thesis, Stockholm University, 1992.
26. King, J.W., *J. Chromatogr. Sci. 27:*355 (1989).
27. Lu, X.J., M.R. Myers and W.E., Artz, *J. Am. Oil Chem. Soc. 70:*355 (1993).
28. Silver, A.H., and H.T. Kalinoski, *Ibid. 69:*599 (1992).
29. Geissler, P.R., and A.E. Johnson Jr., *Ibid. 67:*541 (1990).
30. Chester, T.L., and D.P. Innis, *Abstracts of the 2nd European Symposium on Analytical Supercritical Fluid Chromatography*, Huethig-Verlag, Heidelberg, 1993, pp. 16–26.
31. Pinkston, J.D., T.E.Delaney, D.J. Bowling and T.L. Chester, *J. High Resolut. Chromtogr. 14:*401 (1991).
32. Steiner, J., *INFORM 4:*706 (1993).
33. Liu, J., T. Lee, E. Babik, Jr., M. Guzman-Harty and C. Hastilow, *J. Am. Oil Chem. Soc. 70:*343 (1993).
34. France, J.E., and J.W. King, *J. Assoc. Off. Anal. Chem. 74 (6):*1013 (1991). ■